Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in *Drosophila melanogaster*

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Sensitization to repeated doses of psychostimulants is thought to be an important component underlying the addictive process in humans [1–4]. In all vertebrate animal models, including humans [5], and even in fruit flies, sensitization is observed after repeated exposure to volatilized crack cocaine [6]. In vertebrates, sensitization is thought to be initiated by processes occurring in brain regions that contain dopamine cell bodies [2,7]. Here, we show that modulated cell signaling in the *Drosophila* dopamine and serotonin neurons plays an essential role in cocaine sensitization. Targeted expression of either a stimulatory ($G\alpha_s$) or inhibitory ($G\alpha_i$) Gα subunit, or tetanus toxin light chain (TNT) in dopamine and serotonin neurons of living flies blocked behavioral sensitization to repeated cocaine exposures. These flies showed alterations in their initial cocaine responsiveness that correlated with compensatory adaptations of postsynaptic receptor sensitivity. Finally, repeated drug stimulation of a nerve cord preparation that is postsynaptic to the brain amine cells failed to induce sensitization, further showing the importance of presynaptic modulation in sensitization.

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Results and discussion

Altered cocaine responsiveness in lines expressing $G\alpha_s$, $G\alpha_i$ or *TNT* in dopamine and serotonin neurons

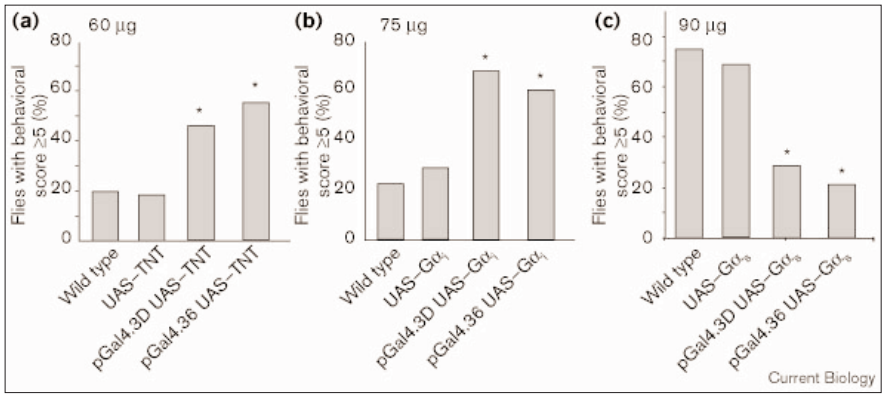
To make a binary system for the targeted expression of gene products in dopamine and serotonin neurons, we generated *pDdc–GAL4* transgenic flies expressing the yeast GAL4 transcriptional activator under the control of the *Ddc* (dopa decarboxylase) promoter (see Materials and methods). Transgenic flies expressing either $G\alpha_i$ or $G\alpha_s$ were generated by crossing flies containing *Ddc–GAL4* with flies expressing either $G\alpha_i$ or $G\alpha_s$ under the control of the UAS, GAL4-responsive site. In vertebrate aminergic neurons, amine synthesis and release are negatively regulated by $G\alpha_i$-coupled autoreceptors [8,9]. If a parallel situation exists in the *Drosophila* central nervous system (CNS), misexpression of $G\alpha_i$ should decrease amine synthesis and release, whereas targeted misexpression of $G\alpha_s$ might compete with the endogenously expressed $G\alpha_i$, resulting in increased amine synthesis and release. As we cannot measure amine release directly in this system, we expressed TNT in these neurons as a control, because expression of TNT in *Drosophila* abolishes evoked synaptic release and partially inhibits spontaneous release [10].

Administration of cocaine to transgenic lines expressing either $G\alpha_i$ or TNT in the dopamine and serotonin neurons resulted in a similar phenotype of hypersensitivity to the initial exposure to cocaine, where hypersensitivity is indicated by an increased fraction of flies showing severe responses to the challenge dose of cocaine (Figure 1a,b). In contrast, fly lines expressing $G\alpha_s$ in these neurons showed the opposite phenotype, that is, reduced responsiveness to cocaine compared with controls (Figure 1c). These results are most simply interpreted as being due to compensatory postsynaptic responses to altered levels of amine release, analogous to the postsynaptic hypersensitivity observed in vertebrates with induced deficits in dopamine release [11,12]. As a control for the effects of the *pDdc–GAL4* driver, we crossed the *pDdc–GAL4* transgenic flies with a line expressing a mutant form of TNT [10]. These flies showed normal responsiveness to a single dose of cocaine, and normal sensitization to repeated doses (data not shown).

Altered postsynaptic functions in flies expressing $G\alpha_s$, $G\alpha_i$ or *TNT*

To determine whether the observed changes in cocaine responsiveness in the fly lines expressing $G\alpha_s$, $G\alpha_i$ or TNT were due to altered postsynaptic function, we examined the sensitivity of receptors activated by the dopamine D2-like agonist quinpirole in the *Drosophila* nerve cord, which contains neurons that are postsynaptic to the brain aminergic neurons. Quinpirole stimulates locomotor responses in decapitated flies [13], the magnitude of this effect presumably reflecting the state of the postsynaptic quinpirole-sensitive receptors before decapitation. Using this behaviorally active preparation, we found that the responsiveness of the nerve-cord receptors correlated with the magnitude of cocaine responsiveness *in vivo*. Both the $G\alpha_i$- and TNT-expressing lines showed significantly increased locomotor responses compared

Figure 1



Alterations in the initial cocaine responsiveness of male flies expressing TNT, $G\alpha_i$ or $G\alpha_s$ in dopamine and serotonin neurons. TNT, $G\alpha_s$ and $G\alpha_i$ were expressed under UAS control using either the *pDdc-GAL4.36* or *pDdc-GAL4.3D* drivers (see Materials and methods). Flies were exposed to volatilized free base cocaine and the responses were scored using a behavioral scoring system [6]. Behavioral scores range from 0 (normal) to 7 (death). The fractions of flies showing severe responses, corresponding to a behavioral score of ≥ 5 (bouts of rapid twirling and erratic jumping or paralysis), at any time during a 5 min viewing period after cocaine exposure are plotted. In each panel, different amounts of cocaine were used to compensate for the altered initial responsiveness. **(a)** TNT-expressing flies and controls were exposed to 60 µg cocaine. **(b)** $G\alpha_i$-expressing flies and controls were exposed to 75 µg cocaine. **(c)** $G\alpha_s$-expressing flies and controls were exposed to 90 µg cocaine. In all panels, the responses of a wild-type (w^{1118}) line and of UAS flies in the absence of a *GAL4* driver are shown as controls. For each strain, 55–120 flies were tested. Asterisks indicate significant differences (Chi squared) in responsiveness compared with the wild-type controls ($p < 0.05$). Additional controls (data not shown) indicate that the nature of the cocaine-induced behaviors is the same in the wild-type and UAS-expressing lines, and that similar results are observed if different behavioral criteria and different cocaine amounts are used.

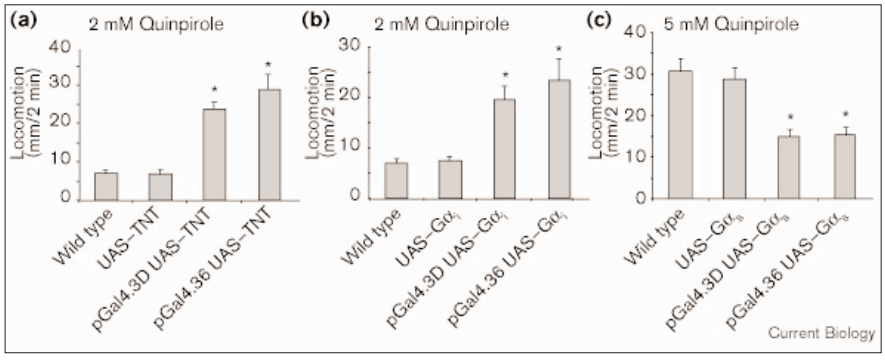
with control lines (Figure 2a,b), whereas $G\alpha_s$-expressing flies showed decreased locomotor responses (Figure 2c). We conclude that long-term deviations in the levels of released amines can be compensated for by reciprocal changes in postsynaptic receptor sensitivity. Furthermore, the sensitivity of these postsynaptic receptors correlates with the magnitude of cocaine responsiveness *in vivo*.

The overall locomotor activity in these lines showing altered cocaine responsiveness appeared normal when assayed in a *Drosophila* activity monitor (data not shown). Thus, the degree of postsynaptic receptor compensation is sufficient to maintain normal homeostasis in the aminergic control of locomotor activity.

Lack of sensitization in lines expressing $G\alpha_s$, $G\alpha_i$ or *TNT* in dopamine and serotonin neurons

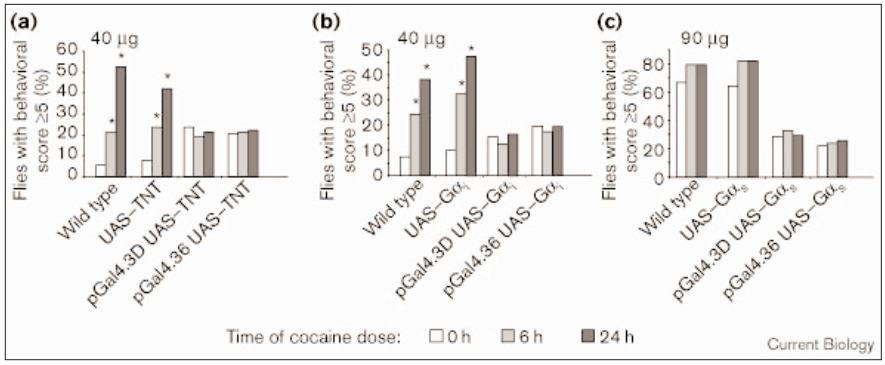
In vertebrates, sensitization appears to result from modulations in aminergic neurons that occur in response to repeated psychostimulant exposure [3,14,15], although the precise nature of these altered functions is not clear. To determine whether expression of $G\alpha_s$, $G\alpha_i$ or TNT in aminergic cells interferes with sensitization, flies expressing these genes in dopamine and serotonin neurons were given three doses of cocaine and behavioral responses were determined after each exposure (Figure 3). Repeated exposures led to robust sensitization in controls, but expression of any of these gene products in the dopamine and serotonin neurons abolished sensitization.

Figure 2



Alterations in the responsiveness of postsynaptic nerve-cord dopamine receptors in flies expressing TNT, $G\alpha_i$ or $G\alpha_s$ in dopamine and serotonin neurons. Locomotor responses stimulated by the D2-like agonist quinpirole were measured as the amount of induced locomotion in a 2 min viewing period after drug administration. In **(a,b)**, 2 mM quinpirole was used, whereas in **(c)**, 5 mM quinpirole was used to compensate for the decreased responsiveness of the $G\alpha_s$-expressing flies. (a) TNT-expressing flies and controls ($n = 45–60$ for each line). (b) $G\alpha_i$-expressing flies and controls ($n = 55–60$ for each control line and 20–35 for each $G\alpha_i$-expressing line). (c) $G\alpha_s$-expressing flies and controls ($n = 50–85$ for each line). In all panels, the responses of a wild-type (w^{1118}) line and of UAS flies in the absence of a *GAL4* driver are shown as controls. Asterisks indicate significant differences (ANOVA) in induced locomotion compared with the wild-type controls ($p < 0.001$).

Figure 3



Expression of $G\alpha_s$, $G\alpha_i$ or TNT in dopamine and serotonin neurons blocks sensitization to repeated cocaine exposures. Male flies expressing these gene products in dopamine and serotonin neurons were exposed to three cocaine doses at 0, 6 and 24 h, and the behavioral responses were determined [6]. The fractions of flies showing severe responses (behavioral score ≥5) in a 5 min scoring episode after each exposure are plotted ($n = 55–125$ for each line). Asterisks indicate significant differences in responses to the first exposure compared with subsequent exposures (Chi squared; $p < 0.01$). **(a,b)** Flies expressing TNT or $G\alpha_i$ and controls were exposed to 40 µg cocaine. **(c)** $G\alpha_s$-expressing flies and controls were exposed to 90 µg cocaine. Cocaine doses were altered to compensate for the different initial responsiveness of the flies expressing the given transgenes. The lack of sensitization in wild-type flies exposed to 90 µg cocaine is due to the near-maximal initial responses to this high dose. Each line expressing an ectopic gene product was tested at additional cocaine exposure levels, but sensitization was never observed (data not shown).

Because ectopic G-protein expression in these presynaptic neurons blocked sensitization, we infer that sensitization requires modulation of G-protein signaling following even a single cocaine exposure, or that the ectopic G-protein expression is sufficient to block other modulations in cell signaling. TNT blocks evoked synaptic release, such that it will block any effects of G-protein modulation of amine release in these neurons. Presumably, there is a small amount of residual non-evoked release in these flies that is sufficient to allow survival, as has been seen previously for glutamate release at the neuromuscular junction after inhibition with tetanus toxin [10].

Misexpression of $G\alpha_s$, $G\alpha_i$ or TNT during development produced neither lethality nor overt behavioral phenotypes, which suggests that perturbations of neurotransmission either through disrupted G-protein signaling or TNT expression do not significantly affect basic neuronal function. Furthermore, confocal images of CNS immunostained for Ddc or serotonin showed grossly normal morphology when $G\alpha_s$, $G\alpha_i$ or TNT were expressed in the dopamine and serotonin neurons (data not shown), suggesting that the observed cocaine phenotypes do not result from developmental abnormalities.

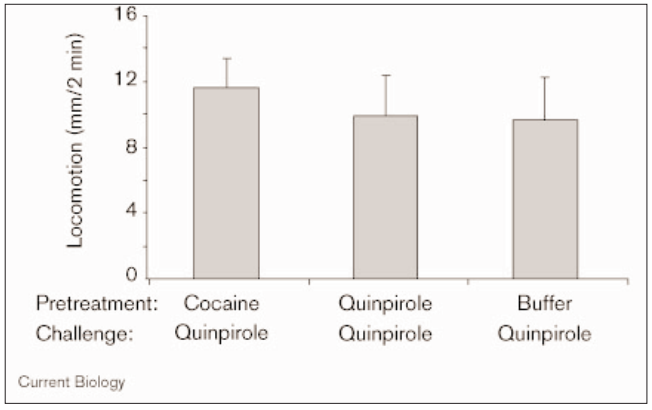
Failure of a nerve cord preparation to sensitize

Sensitization in vertebrates can be elicited by direct injection of psychostimulants into the dopamine-cell-rich ventral tegmental area [15], but injections into the region to which the ventral tegmental neurons project, the nucleus accumbens, fail to result in sensitization (reviewed in [14]). Sensitization results in long-lasting hypersensitivity of the postsynaptic receptors, both in vertebrates [2,16] and in *Drosophila*. When living flies are sensitized by repeated exposures to cocaine and subsequently decapitated, the decapitated preparations show increased responsiveness when challenged with quinpirole [17]. In contrast, exposing decapitated preparations of drug-naive flies to cocaine or quinpirole failed to induce hypersensitive responses to a subsequent dose of quinpirole (Figure 4). These results show further parallels with results in vertebrates, in that the development of sensitization critically requires modulation in aminergic cells. However, repeated cocaine or dopamine agonist exposures of this nerve cord preparation, which is postsynaptic to the brain aminergic neurons, is not sufficient to yield sensitization.

Cocaine responsiveness correlates with compensatory adaptations of postsynaptic responsiveness

The results presented here unambiguously show critical roles for the biogenic amines dopamine and/or serotonin in modulating cocaine responsiveness and sensitization. The

Figure 4



Repeated stimulation of the nerve cord with cocaine or the dopamine agonist quinpirole fails to induce sensitization. Drugs were applied to the nerve cords of decapitated male flies (0.05 mg/ml cocaine–HCl, 2 mM quinpirole, or buffer), which were challenged with 2 mM quinpirole 6 h later. Locomotion following quinpirole addition was assayed in a 2 min viewing period. Average locomotion ± SEM is shown ($n = 15–21$).

directionality of the alterations in cocaine responsiveness observed is somewhat counterintuitive; long-term expression of gene products that are expected to reduce amine release increased responsiveness to cocaine, whereas expression of gene products expected to increase amine release decreased responsiveness. These results are consistent with the measured changes in the responsiveness of the nerve cord dopamine receptors, which are in a compensatory direction to the expected alterations in the level of release. These compensatory neuroadaptations have parallels in several situations in vertebrates: animal models that have been amine-depleted with reserpine show sensitized responsiveness of postsynaptic amine receptors [18,19], as do animals with unilateral dopamine cell ablations [12]. Similarly, in the clinical setting, postsynaptic dopamine receptor modulation is thought to complicate long-term treatment of Parkinson's patients with L-DOPA [20,21]. Similar compensatory postsynaptic changes are observed in mice heterozygous for loss of VMAT2 [11,22], the primary vesicular monoamine transporter in the brain. In parallel with our results in *Drosophila*, these animals show no additional sensitized response when repeatedly exposed to amphetamine [11]. This indicates the determining impact of presynaptic alterations in amine release on postsynaptic receptor responsiveness.

We note a concurrent report in this issue [23] showing that treatment of *Drosophila* with a dopamine synthesis inhibitor that reduces whole fly dopamine levels ~tenfold leads to reduced responses to cocaine, the opposite of our findings. It remains to be elucidated whether this discrepancy results from differences in the time courses of the pharmacological versus developmental approaches, from differences in pharmacological specificity, or from differences in amounts of inhibition of amine release.

Supplementary material

Additional results and discussion and additional methodological details are available at http://current-biology.com/supmat/supmatin.htm.

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